TRANSLATION NO. 1647

DATE: Jan 1961

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

#1047

two cases of rabies were recorded in all, and in Denmark, Austria and the Nederlands no cases of this infection were recorded.

The present registration of such zoonoses as Q-fever, leptospirosis and psittacosis (ornithosis) does not guarantee a true presentation of the distribution of these little-studied infections. According to the data of the World Health Organization, a few cases and small outbreaks of Q-fever were recorded in the U.S.A. (4–63 cases), Western Germany (?–137 cases), Italy (9–165 cases), Portugal (1–5 cases) and Switzerland (17–19 cases). Leptospirosis (icterohaemorrhagic) was recorded annually in every continent with a small distribution. The highest incidence was observed in Yugoslavia (1032–1037 cases per annum). A few cases and small outbreaks of psittacosis were recorded annually only in Southern Asiatic countries and in the U.S.A. where the largest number of cases (368) was observed in 1956.

By comparing the data in the distribution of zoonoses in the past and in the present, we can draw the conclusion that the terrible scourge of mankind—plague—appears at the present time in the form of focal epidemic outbreaks and sporadic cases in those countries with natural foci of this infection. The possibility of extensive epidemics and pandemics is excluded by modern methods of combating plague.

Brucellosis, which has assumed the character of enzootics and as yet has shown no tendency to decrease, continues to represent a serious threat to the health of the population and to the development of stock-breeding.

The diseases of rabies and tetanus continue to cause the loss of many human lives, especially in colonial and dependent countries where there is also a considerable distribution of anthrax.

The spread of zoonoses into the U.S.S.R. from abroad is not excluded, in connexion with which measures for sanitary protection remains one of the current tasks of Health Organizations.

Translated by F. S. Freisinger

CURRENT STUDIES OF MELIOIDOSIS AND CERTAIN TASKS FOR SCIENTIFIC INVESTIGATION*

M. I. Levi

The Caucasus and Trans-Caucasus Anti-Plague Research Institute (Stavropol)

(*Received* 24 *June* 1959)

In 1912 Whitmore the morbid-anatomist of the main hospital in Rangoon, described some dozens of cases of a new disease which was subsequently called melioidosis (pseudo-glanders). A new organism was isolated from the internal organs of patients who died from this disease. It appeared as a bipolar staining bacillus similar in morphology to *mallei*. The bacillus was motile. It grew quickly and abundantly under aerobic conditions in the normal nutrient media and in broth a thick, wrinkled film was formed, on glycerol agar (4–5% agar) there appeared cream-coloured wrinkled colonies. Whitmore's bacillus (*malleomyces pseudomallei*) caused the death of guinea-pigs when

* Zh. mikrobiol. epidemiol. immunobiol. 31: No. 2, 133–139, 1960.

injected by various routes. The animals died after several days. When males were injected intraperitoneally with the organisms, swelling of the scrotum and inflammation of the tissue of the testes was observed which did not spread to the peritoneal cavity.

Whitmore discovered strains of the causative organism of the new disease in England, where in the laboratory of Dr. Ayre (Liverpool) his observations and conclusions were confirmed.

A great amount of work on the new disease was carried out from 1921–1925 by Stanton and Fletcher in Malaya. They not only isolated new strains of the causative organism from patients, but also demonstrated the possibility

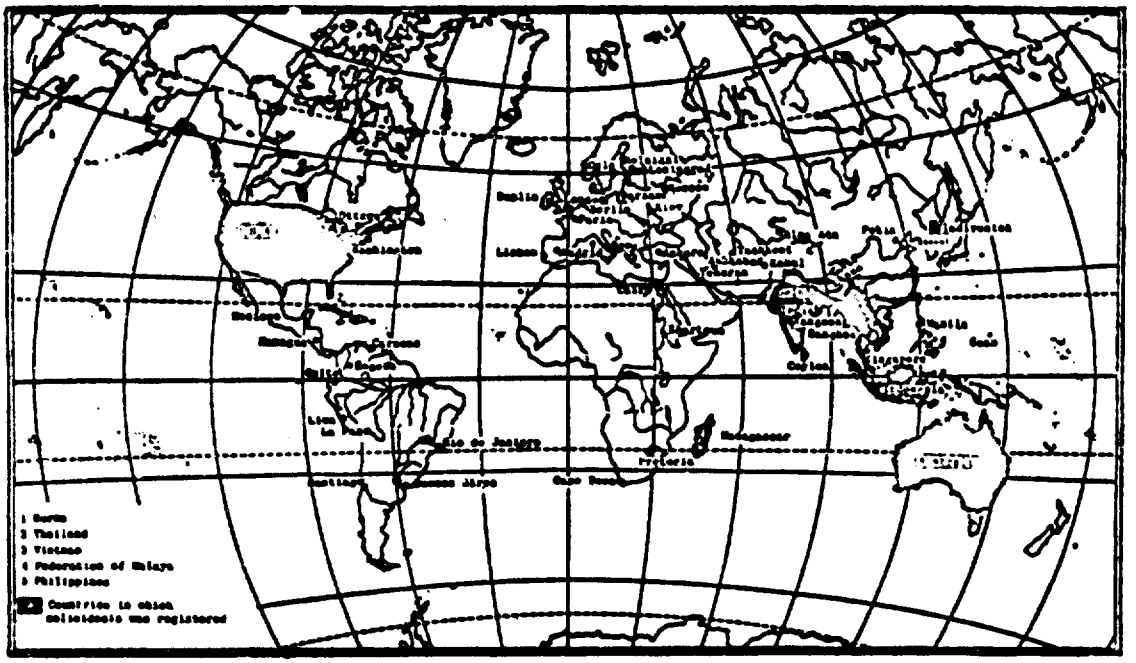

FIG. 1. The geographical distribution of melioidosis throughout the world.

of the spontaneous infection of laboratory animals—guinea-pigs and rabbits. The bacilli could also be found in certain wild rats (*Mus griseiventer*). Stanton and Fletcher observed a great similarity between the Whitmore bacillus and *Pasteurella pestis* but the motility of the *M. pseudomallei* of melioidosis and its ability to form distinctive wrinkled (corrugated) colonies on glycerol agar, make it possible to differentiate it not only from *P. pestis* but also from *M. mallei.*

In subsequent years numerous cases of human infections with melioidosis were recorded in various countries, chiefly in South-East Asia—Burma, India, Malaya, Vietnam, Singapore, Indonesia, Thailand, Ceylon, Australia, the Philippines, Guam, Madagascar and the U.S.A. (see map).

In the Soviet Union cases of melioidosis were not observed. Papers which

appeared in the literature by Imamaliev and Livshits, and also by Guseinov on a case of melioidosis in Azerbaidzhan were not confirmed when investigated.

Among Soviet investigators, Lutsenko in 1931 first carried aut a comparative study of a number of the properties of the *M. mallei* and *M. pseudomallei*. In 1941 Chalisov's survey of the literature on melioidosis was published.

Similar work was published in 1956 by Popov. In 1957 Lebedinskii, Gapochko and Garin presented a short survey of the epidemiology, clinical details and prophylaxis of melioidosis.

At present acute and chronic forms of melioidosis are differentiated. It has been established that the former can take a hyper-acute and sub-acute form.

The distinctive features of the acute forms of melioidosis are septicemia and toxemia, high fever, foci of inflammation in the lungs, enlargement of the liver and spleen, leukocytosis and a high erythrocyte sedimentation rate. Cases of hyper-acute and acute septic melioidosis usually result in the death of the patient. The marked frequency of illnesses with involvement of the lungs attracts attention.

Chronic melioidosis usually pursues a course with suppurative abscesses in the subcutaneous tissue, bones and internal organs, frequently with fistulae which do not heal for a long time. If a case of melioidosis can be suspected on the basis of clinical indications, a final diagnosis is possible only by means of bacteriological methods.

Recently, cases of the recovery of patients from melioidosis have been described after intensive treatment with chloramphenicol (the concentration of antibiotic in the blood must reach 20–30 μg/ml.). Other antibiotics have proved less effective. Strains resistant to chloramphenicol have been detected. In these cases treatment should be carried out with chlortetracycline and oxytetracycline (Brygoo and Henry; Chambon, de Lajoudie and Fournier; Chambon).

In chronic melioidosis surgical intervention, as well as antibacterial therapy is of decisive importance—excision and ablation of infected regions and purulent foci (Peck and Tsvaenberg).

The best method of diagnosing melioidosis is the isolation of the causative organisms, *M. pseudomallei*. This is a gram-negative bacillus 0·5–1 μ in size with 1–4 terminal flagella (Brindle and Cowan; Brygoo; de Lajoudie, Fournier and Chambon).

M. pseudomallei is similar to *M. mallei* and also to *Pseudomonas pyocyanea*. The most characteristic differential features of *M. pseudomallei* are its motility, the formation of characteristic colonies with a corrugated surface on glycerol agar, and the absence of the ability to produce a blue-green pigment which diffuses into the nutrient medium. It is known that *M. mallei.* is non-motile, while *Ps. pyocyanea* does not form such dry and corrugated colonies on glycerol agar and usually liberates a blue-green pigment into the surrounding medium.

Colonies of *M. pseudomallei* can have the smooth or rough form. It is assumed that the virulence of the culture is conditioned by organisms in the R-form (Miller *et al.*).

M. pseudomallei relatively quickly causes liquifaction of gelatin, in broth it forms a thick film, on a potato it grows in the form of coffee-coloured, yellow or cream-coloured film. The culture gives off a distinctive aromatic smell. The organism ferments various sugars and forms acid without gas, and sulphide and indol gases are not formed.

Freshly isolated strains of *M. pseudomallei* are pathogenic for wild rats, cats, dogs, guinea-pigs, hamsters, white mice, white rats and monkeys. For laboratory work, white mice, guinea-pigs and hamsters are commonly used.

When male guinea-pigs are injected intraperitoneally with suspected material, a broth culture or a suspension of an agar culture the Strauss phenomenon develops (erythema and acute oedema of the testes). It should be mentioned that *M. mallei* also evokes the Strauss phenomenon. Subcutaneous injection of a culture into guinea-pigs is accompanied by the appearance of a necrotic zone, followed by an ulcer covered with a purulent coating. There are found numerous purulent foci in the liver, lungs and spleen. The latter are true infectious granulomata at various stages of development, and usually have in the centre, necrotic regions surrounded by granulation tissue.

M. pseudomallei contains a toxic material which apparently causes the rapid death in hyper-acute melioidosis in man and experimental animals (Nigg, Heckly and Colling).

The somatic antigen of *M. pseudomallei* is similar in structure to the somatic antigen of *M. mallei* but the latter lacks a flagellar antigen. Among the strains of *M. mallei* there are some with an atigenic structure similar to that of *M. pseudomallei* but there are some which are very different (Stanton and Fletcher).

The serological diagnosis of melioidosis has been insufficiently developed, but by applying certain serological methods it is possible to obtain an entirely satisfactory result. The complement-fixation test is of the greatest diagnostic importance, and a definite part can be played by the passive haemagglutination test of the Kravchenko-Sokolov of Middelbrook-Dubos type. The simplest to use is the agglutination test, but its titre can be considered diagnostic only from 640 and higher (Cravitz and Miller; Brygoo, Fournier, de Lajoudie and Chambon).

It follows from everything said so far that only the detection of *M. pseudomallei* has diagnostic significance. The latter can be identified in the material under investigation (purulent secretion, sputum, etc.), by the use of fluorescent anti-sera (Moodie, Tomson and Goldman; Tomson, Moodie and Goldman). This method makes possible the rapid detection of the organism both in material from patients and in the external environment (it must be taken

into account that when using a fluorescent immune serum it is impossible to differentiate between *M. pseudomallei* and *M. mallei*). If the organism is isolated by the usual bacteriological method most importance is attached to the nature of the growth on glycerol agar, its pathogenicity for laboratory animals, and for male guinea-pigs in particular, and the motility. Immunological investigation of the isolated strain with both fluorescent anti-sera and the above serological tests completes the identification of the organism.

Serological methods of investigating the sera of patients although they take second place in detecting the organism, play an important part in the diagnosis of subacute and chronic cases.

Epidemiological problems of melioidosis have been investigated so slightly that it is difficult to systematize the known facts in this field.

The source of infection in man is unknown. Cases of human infection from patients with melioidosis have not been recorded neither are hospital or laboratory cases. Already in the 1920's Stanton and Fletcher found infected wild rats, cats and dogs in nature (the Malacca peninsula), and horses infected with melioidosis were detected in Indonesia (Stanton, Fletcher and Simmonds). Gerard isolated *M. pseudomallei* from pigs in Madagascar and Lewis and Olds recorded melioidosis in sheep and goats in Australia. In investigation tens of thousands of rats in South Vietnam and Burma negative results were obtained. *M. pseudomallei* and a specific phage to it were isolated many times from the silt and water of various reservoirs in South Vietnam. There are known cases of the infection of persons who used muddy water and were infected by cooked food. This is assisted by the fact that *M. pseudomallei* is very resistant to the effect of the external environment and, in particular, it can remain alive in water for several months. Under experimental conditions it is easy to produce infection in laboratory animals by giving them infected food and drinking water.

Regarding the fact that it has not been possible in every country to confirm the rôle of rats as a reservoir of infection for man, deratization measures should be carried out only where the significance of these animals in the epidemiology of the disease is definitely established.

Disinfection measures are carried out with a 1% solution of potassium permanganate, a 1–5% solution of phenol or with the usual iodine tincture, which quickly destroy *M. pseudomallei*.

Thus, melioidosis belongs to the septico-toxemic diseases of the plague and anthrax type and is highly lethal. The distinguishing feature of *M. pseudomallei* is its very great resistance in the external environment, its high pathogenicity for laboratory animals on aerogenic infection (in this case a few organism are sufficient for infection). No prophylaxis of melioidosis has been developed. Only one antibiotic (chloramphenicol) is used in treatment, and this is effective in by no means every case.

In certain American laboratories, especially in those situated in Camp Detrick, a number of eminent scientists are engaged in the study of the possibility of using *M. pseudomallei* as a means of bacteriological warfare. In his book *"Peace or Plague"* Roseberry, and several other American authors propose that the "destructive power" of *M. pseudomallei* is roughly the same as that of *P. pestis.*

All the above indicates the necessity of a sharp intensification of research into the prophylaxis treatment and bacteriological diagnosis of melioidosis. It seems to us that this work should be carried out in the following directions:

(1) It is necessary to search for the possibility of the rapid diagnosis of melioidosis by means of fluorescent immune sera. Methods of preparing such anti-sera have been developed. By using specific fluorescent anti-sera it is possible to detect *M. pseudomallei* not only in mucus or purulent secretions, but also in external media (food products, soil, water, air). This method makes it possible to detect *M. pseudomallei* even in mixed cultures, although *M. pseudomallei* cannot be differentiated from *M. mallei.* In the near future the production of fluorescent immune sera mist be organized and all interested establishments should be supplied with them.

(2) Research must be carried out to elaborate methods of active immunization. At the present time it is known that a number of old laboratory strains have lost their pathogenicity for animals. We are faced with a large amount of work in the study of the possibility of using avirulent strains of *M. pseudomallei* for active immunization. In the recently published work of Dannenberg and Scott, which the authors state to be the first in a series of papers, it is mentioned that the third communication will be devoted to the investigation of avirulent strains as a live vaccine.

Of no lesser interest is the problem of preparing a chemical vaccine against melioidosis. It should be mentioned that questions of the antigenic structure of *M. pseudomallei* and the immunogenic properties of the various antigens have clearly been insufficiently studied. These questions required urgent solution, since they are connected not only with the problem of preparing a chemical vaccine, but also with questions of the improvement of the specific diagnosis of melioidosis.

In nearly all recent papers by American authors chief attention has been paid to the aerogenic method of infecting experimental animals. In connexion with this, the immunity of vaccinated animals must be investigated under conditions of aerogenic as well as of parenteral infection. Laboratories must be provided with proper apparatus for obtaining resistant suspension of bacteria in air and apparatus for making an objective count of the concentration of bacteria in this medium.

American investigators have developed a method for rapidly increasing the virulence of *M. pseudomallei.* As well as confirming these findings, methods for reducing the virulence of strains must be studied.

(3) The task of quickly destroying *M. pseudomallei* in the external environment is of great interest, especially in air and water, with the aid of the normal disinfection measures. In carrying out this work those agents which possess the widest range of effect must be studied first.

(4) Problems of treatment are of particular interest not only to clinicians but also to bacteriologists. In this project investigators are presented with the following tasks: (a) to test the effect of various methods of infection including the aerogenic method; (b) to develop methods of preparing specific anti-sera and testing their effectiveness both alone and in combination with antibiotics; (c) to study the possibility of phage therapy; (d) to study the mechanism of the development of the rate of formation of antibiotics—resistant forms of the organisms; (e) the therapy of experimental melioidosis complicated by the effect of penetrating radiation on animals.

To study problems of the treatment successfully it is necessary to have a laboratory model of the chronic form of melioidosis.

(5) In order to obtain a large amount of bacterial suspension rapidly, it is necessary to develop a method of deep cultivation of *M. pseudomallei.* According to the data of the American authors Miller, Pannell, Cravitz and Ingolls (the research was carried out in Camp Detrick), by this method cultures can be obtained with a high concentration of organism (2×10^{10}/ml). Particular attention must be paid here to the selection of nutrient media suitable for cultivation and of the conditions of aeration.

(6) Research must also be carried out to obtain and study strains of specific phage in order to use it for the rapid identification of *M. pseudomallei,* to isolate the toxin of *M. mallei* and to study its chemical nature and toxigenic properties, the possibility of obtaining an antitoxic immune serum, and detection of the toxin in the external environment, e.g. in water, and to study the possibility of transforming the toxin into toxoid.

(7) The pathogenesis of experimental melioidosis when laboratory animals are infected by various methods must be studied, and also in certain species of domestic and wild animals, and the period of excretion of the organism into the environment.

(8) Of great importance for the further development of knowledge concerning melioidosis is the question of the position of the organisms in the system of micro-organisms, the similarity and differences between *M. pseudomallei* and *M. mallei, P. pestis, B. anthracis* and various strains of *Ps. pyocyanea, Salmonella* and other organisms.

(9) Finally, undoubted importance is attached to the question of the distribution of melioidosis in countries in immediate proximity to the borders of the Soviet Union, and geographical distribution of melioidosis throughout the world.

Translated by F. S. FREISINGER

REFERENCES

GUSEINOV, D. Iu., Tezisy dokl. konferentsii patologoanatomov respublik Zakavkaz'ia po problemam krayevoi patologii. (Summaries of Reports of the Conference of Morbid Anatomists of the Transcaucasian Republics on Problems of Regional Pathology.) 14, Baku, 1957

IMAMALIYEV, S. A. and LIVSHITS, A. A., Tezisy dokl. konferentsii patologoanatomov respublik Zakavkaz'ia po problemam krayevoi patologii. (Summaries of Reports of the Conference of Morbid Anatomists of the Transcaucasian Republics on Problems of Regional Pathology.) 15, Baku, 1957

LEBYEDINSKII, V. A., GAPOCHKO, K. G. and GARIN, N. S., Voen.-med. zh. No. 1, 72, 1957

LUTSENKO, T. A., Prakt. veterinariia No. 8–9, 703, 1930

LUTSENKO, T. A., Veterinarne dilo No. 2, 18, 1930

LUTSENKO, T. A., Labor, praktika No. 5, 6, 1931

POPOV, K. N., Zh. mikrobiol. epidemiol. immunobiol. No. 1, 125, 1957

CHALISOV, I. A., Zh. mikrobiol. epidemiol. immunobiol. No. 12, 3, 1941

BEAMER, P. R., VARNEY, P. L., BROWN, W. G. *et al.*, Am. J. Clin. Path. 24: 1231, 1954

BRINDLE, C. S. and COWANS, S. T., J. Path. Bact. 63: 571, 1951

BRYGOO, E. R. and HENRY, E., Bull. Soc. path. exotique 46: 279, 1953

BRYGOO, E. R., Bull. Soc. path. exotique 46: 347, 1953

BRYGOO, E. R., Ann. Inst. Pasteur 92: 688, 1957

CHAMBON, L., de LAJUDIE, P. and FOURNIER, J., Bull. Soc. path. exotique 43: 139, 1954

CHAMBON, L., Ann. Inst. Pasteur 89: 229, 1955

CHAMBON, L., Ann. Inst. Pasteur 88: 315, 1955

CRAVITZ, L. and MILLER, W. R., J. Infect. Dis. 86: 46, 1950

DANNENBERG, A. M. and SCOTT, E. M., J. Exp. Med. 107: 153, 1958

FOURNIER, J., de LAJUDIE, P. and CHAMBON, L., Med. tropicale 13: 683, 1953

GIRARD, G., Bull. Soc. path. exotique 29: 712, 1936

de LAJUDIE, P., FOURNIER, J. and CHAMBON, L., Ann. Inst. Pasteur 85: 112, 1953

LEWIS, F. A. and OLDS, R. J., Australian Vet. J. 28: 145, 1952

MILLER, W., PANNELL, Z., CRAVITZ, L. *et al.*, J. Bact. 55: 115, 1948

MILLER, W., PANNELL, L., CRAVITZ, L. *et al.*, J. Bact. 55: 127, 1948

MIRICK, G. S., ZIMMERMAN, H. M., MANER, G. D. *et al.*, J.A.M.A. 130: 1063, 1946

MOODY, M. D., GOLDMAN, M. and THOMASON, B. M., J. Bact. 72: 357, 1956

NIGG, C., HECKLY, R. and COLLING, M., Proc. Soc. Exp. Biol. Med. 89: 17, 1955

PATON, J. P. J., PECK, C. R. and SCHAAF, A. VAN DE, Brit. M. J. 1: 336, 1947

STANTON, A. T., FLETCHER, W. and SYMONDS, S. L., J. Hyg. 26: 33. 1927

STANTON, A. T. and FLETCHER, W., J. Hyg. 23: 347, 1925

STANTON, A. J., FLETCHER, W. and KANAGARAYER, K., J. Hyg. 23: 268, 1924–1925

THOMASON, M., MOODY, M. D. and GOLDMAN, M. J. Bact. 72: 362, 1956

TOPLEY and WILSON, Principles of Bacteriology and Immunity. London, 1955